Claims

1. A method for performing a qualitative or quantitative assay for protein oxidation, comprising detecting in a sample an amino acid sequence which is characteristic of a specific protein and which contains one or more aromatic amino acid residues in nitrated form.

2. A method as claimed in claim 1, conducted as an immunoassay, comprising detecting binding of an immunological binding partner which is immunoreactive with said nitrated form of said aromatic amino acid residue.

3. A method as claimed in claim 2, wherein said immunological binding partner is specifically reactive with said nitrated form of said aromatic amino acid residue in the context of said amino acid sequence which is characteristic of a specific protein.

4. A method as claimed in claim 2, wherein said immunological binding partner is reactive with said nitrated form of said aromatic amino acid residue in a context independent manner and said assay comprises detecting binding of both said immunological binding partner and a second immunological binding partner to nitrated amino acid sequences in a sandwich format, wherein said second immunological binding partner has binding

specificity for an amino acid sequence which is characteristic of said specific protein.

5. A method as claimed in any preceding claim, wherein said specific protein of which the detected nitrated amino acid sequence is detected is a mammalian protein.

6. A method as claimed in claim 5, wherein said protein is present in joint tissue.

7. A method as claimed in claim 6, wherein said protein is collagen of type I, II, III, VI, IX or XI, aggrecan, cartilage link protein, cartilage oligomeric protein, or cartilage intermediate layer protein.

8. A method as claimed in any preceding claim, wherein said nitrated aromatic amino acid residue or residues is/are nitrotyrosine or nitrotryptophan.

9. A method as claimed in claim 8, wherein the amino acid sequence which is detected comprises the sequence HRGYPGLDG in which the amino acid residue Y is nitrated tyrosine or is comprised within said sequence and includes said nitrated tyrosine.

10. A method as claimed in claim 8, wherein the amino acid sequence which is detected comprises the sequence LQYMRA in which the amino acid residue Y

is nitrated tyrosine or is comprised within said sequence and includes said nitrated tyrosine.

11. An immunological binding partner specifically reactive with the nitrated form of an aromatic amino acid residue in the context of an amino acid sequence which is characteristic of a specific protein.

12. An immunological binding partner as claimed in claim 11 having binding specificity for an epitope contained in the amino acid sequence HRGY:NO_2PGLDG which epitope contains the amino acid residue Y:NO_2 and is characteristic of collagen type II.

13. An immunological binding partner as claimed in claim 12, which is an antibody raised against a peptide having the sequence $(X_{aa})_m$HRGY:NO_2PGLDG$(X_{aa})_n$, wherein X denotes any amino acid or derivative thereof and m and n are independent integers of from 1 to 10, or is a fragment of such an antibody.

14. An immunological binding partner as claimed in claim 11, having binding specificity for an epitope contained in the amino acid sequence LQY:NO_2MRA which epitope contains the amino acid residue Y:NO_2 and is characteristic of collagen type II.

15. An immunological binding partner as claimed in claim 14, which is an antibody raised against a peptide having the sequence $(X_{aa})_m$LQY:NO_2MRA$(X_{aa})_n$, wherein X

denotes any amino acid or derivative thereof and m and n are independent integers of from 1 to 10, or is a fragment of such an antibody.

16. An immunological binding partner as claimed in any one of claims 11 to 15, which is a monoclonal antibody or fragment thereof.

17. A cell line producing a monoclonal antibody or fragment thereof as claimed in claim 16.

18. A method for the investigation of the existence or extent of a pathological state comprising measuring in a biological sample the relative amounts of nitrated an non-nitrated forms of an amino acid sequence which is characteristic of a specific protein and which contains one or more nitratable aromatic amino acid residues.

19. A method as claimed in claim 18, wherein the pathological state is oxidative damage associated with an inflammatory joint disease and said specific protein is derived from cartilage matrix.

20. A method as claimed in claim 18, wherein said pathological state is a cancer, Alzheimer's disease, Parkinson's disease, an inflammatory bowel disease, systemic lupus erythematosus, osteoarthritis or rheumatoid arthritis.

21. A method as claimed in any one of claims 18 to 20, comprising:
contacting a biological sample from an individual or a portion of such a sample with a first immunological binding partner which binds said nitrated form of said amino acid sequence and quantitatively determining said binding;
contacting the biological sample or a portion thereof with a second immunological binding partner which binds said non-nitrated form of said amino acid sequence and quantitatively determining said binding;
determining a ratio between said determinations to provide a ratio of the relative amounts of said nitrated and non-nitrated forms of said sequence present in the sample;
an comparing the measured value with values characteristic of healthy individuals or individuals of known pathology.

22. A method as claimed in claim 21, wherein said first immunological binding partner is as claimed in any one of claims 11 to 16.

23. A kit for use in performing a method as claimed in any one of claims 1 to 3 , 5 to 10 or 18 to 22 and comprising:
an immunological binding partner which is specifically reactive with the nitrated form of an aromatic amino acid residue in the context of an

amino acid sequence which is characteristic of a specific protein; and

means for detecting binding of said binding partner and said protein or a fragment thereof.

24. A kit as claimed in claim 23, wherein the immunological binding partner is as claimed in any one of claims 11 to 16.

25. A kit as claimed in claim 23 or claim 24, including an immunological binding partner which is reactive with the said amino acid sequence in non-nitrated form.

26. A kit as claimed in any one of claims 23 to 25, comprising a peptide reactive with a said immunological binding partner.